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Salmonella typhimurium secreted invasion determinants are homologous to *Shigella* Ipa proteins

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Summary

Salmonella typhimurium **secreted proteins (Ssp) were previously implicated in epithelial cell invasion. Here we describe four genes (*sspB*, *sspC*, *sspD*, and *sspA*), located between *spaT* and *prgH*, which encode proteins of 63, 42, 36, and 87 kDa, respectively. These Ssp are homologous to *Shigella flexneri* secreted proteins IpaB, IpaC, IpaD and IpaA. A non-invasive mutant with a transposon insertion in *sspC* lacks Ssp of 87, 42 and 36 kDa. Complementation analyses show that *sspC* and *sspD* encode the 42 and the 36 kDa Ssp, while the 87 kDa Ssp is encoded by *sspA*. *sspC* and *sspD*, but not *sspA*, are required for invasion. Amino-terminal sequencing shows that SspC and SspA are secreted without amino-terminal processing. We further demonstrate that Ssp secretion requires proteins encoded by *prgHIJK*, homologous to the *Shigella* Ipa secretion system, since SspA is abundantly secreted by wild-type bacteria but is completely retained within the cellular fraction of a *prgHIJK* mutant. A precipitate containing abundant SspC and three other major Ssp of 63, 59 and 22 kDa was isolated from culture supernatants of wild-type bacteria. These data indicate that major secreted invasion determinants of *S. typhimurium* are structurally and functionally homolgous to *S. flexneri* Ipa proteins.**

Introduction

A number of enteroinvasive bacterial pathogens secrete virulence determinants which facilitate colonization of their mammalian hosts and evasion of immunological defences (Bliska *et al.*, 1993). Enteroinvasive *Shigella* species secrete three proteins (IpaB, IpaC and IpaD) necessary for the invasion of non-phagocytic epithelial cells (Ménard *et al.*, 1993), a process thought to allow bacteria to invade the basolateral surface of the intestinal epithelium and to spread from cell to cell within the intestine (Perdomo *et al.*, 1994; Zychlinsky *et al.*, 1994b). In addition, IpaB facilitates escape of the bacteria from the phagocytic vacuole (High *et al.*, 1992) and induces apoptosis in macrophages (Zychlinsky *et al.*, 1994a). The Yop proteins secreted by enteropathogenic *Yersinia* spp. have a wide variety of effects on host cells and have tyrosine phosphatase and serine/threonine phosphokinase enzymatic activities. Yops exhibit cytotoxicity, inhibit bacterial phagocytosis by macrophages (Rosqvist *et al.*, 1990), inhibit the macrophage antimicrobial oxidative burst (Bliska and Black, 1995) and suppress production of the cytokine TNFα by macrophages (Beuscher *et al.*, 1995).

Although Yop and Ipa proteins are neither structurally nor functionally homologous, highly related machineries of a *sec*-independent type III secretory pathway (Van Gijsegem *et al.*, 1993) are utilized by *Yersinia* and *Shigella* spp. for Yop and Ipa secretion (Groisman and Ochman, 1993; Straley *et al.*, 1993). Homologues of this secretion apparatus are not restricted to mammalian bacterial pathogens. They are also important to secretion of virulence factors by bacterial plant pathogens and to flagellar assembly by both Gram-negative and Gram-positive bacteria (Shapiro, 1995; Van Gijsegem *et al.*, 1995). The *Shigella flexneri* (*mxi* and *spa*) and *Yersinia* spp. (*ysc*) genes that encode this secretion apparatus are localized on large virulence plasmids (Forsberg *et al.*, 1994; Sasakawa *et al.*, 1992). The Yop and Ipa proteins lack typical signal sequences and utilize additional factors, which may function as chaperones, for their secretion and/or presecretory stabilization. A number of Yops each require an individual chaperone for their secretion (Wattiau *et al.*, 1994). In contrast, IpaB and IpaC are associated with a single small cytoplasmic chaperone, IpgC, which protects these proteins from intracellular degradation. After secretion, IpaB and IpaC form a complex which contains an additional 72 kDa protein, probably IpaA, with unknown function (Ménard *et al.*, 1994b). In addition, IpaB and IpaD have been shown to transiently interact in the bacterial membrane. It has been suggested that this interaction is important for Ipa secretion upon contact with host cells (Ménard *et al.*, 1994a).

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